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Our working hypothesis is that induced p120 loss in the breast will impair E-cadherin function leading to (1) severe adverse consequences to lobular-alveolar development, and (2) positive effects on tumorigenesis or tumor progression leading to increased invasion and metastasis. To determine the role of p120 inactivation in breast cancer, we have proposed to study the consequences of targeted p120 loss of function in the mammary glands of normal and transgenic mouse models for tumorigenesis and metastasis. The first aim proposes to use gene targeting to incorporate loxP sites at strategic locations in the p120 gene such that Cre recombinase-induced deletion of the intervening sequence will inactivate p120. Mice containing this conditional allele ($p120^{flox}$) will be generated and crossed with WAP-Cre mice to target the conditional deletion to the breast. Our major progress in this reporting period is the completion of the targeting construct, a key objective that required sequencing the p120 genomic DNA, inserting lox p sites, and subcloning to the appropropriate tranfection vector. Thus, we can now move forward with generation of the p120 knockout mouse. The mouse will be invaluable for studying further the role of p120 in metastasis and breast cancer.

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## INTRODUCTION

Accumulating evidence indicates that defects in the cytoplasmic mediators of cadherin function, the catenins, may account for cadherin dysfunction in carcinomas where E-cadherin expression is apparently normal. To determine the role of p120-catenin inactivation in breast cancer, we are studying the consequences of targeted p120 loss of function in the mammary glands of normal and transgenic mouse models for tumorigenesis and metastasis. We have used gene targeting to incorporate loxP sites at strategic locations in the p120 gene such that Cre recombinase-induced deletion of the intervening sequence will inactivate p120. Mice containing this conditional allele ($p120^{flox}$) will be generated and crossed with WAP-Cre mice to target the conditional deletion to the breast. We will determine the effects of selective p120 loss in the breast with respect to lobular-alveolar development, cadherin function, and tumor progression and metastasis. Our working hypothesis is that induced p120 loss in the breast will impair E-cadherin function leading to (1) severe adverse consequences to lobular-alveolar development, and (2) an acceleration of tumorigenesis or tumor progression leading to increased invasion and metastasis. Understanding the role of p120 in these processes may lead to new strategies for pharmacologic intervention as a means of inhibiting metatasis.

## BODY

The statement of work for this grant is included below for reference. We have completed Task 1, the prerequisite work for generating the conditional knockout mouse. Essentially, we completely sequenced ~8 kilobases of the cloned p120 genomic DNA surrounding the sequence to be deleted and generated a detailed restriction map. Using this information, sites were selected to insert the lox sites in introns that flank thep120 start sites. Loss of the intervening sequence removes the initial and internal ATG start sites, and is expected to eliminate p120 gene transcription. The resulting DNA was subcloned into a vector that introduces a neo cassette, for use in selection of transfected ES cells. Our only modification of the original scheme is the insertion of frt motifs on either side of the neo cassette. In some instances, unscheduled splicing into the neo construct has been problematic for others. The modification makes is possible to remove neo from recombined p120 gene before moving the ES cells into mice.

We are just beginning the experiments proposed in Task 2. Essentially, this involves generating the ES cell lines by stable transfection of our targeting construct. As illustrated by our time table, we are pretty much on target and should be ready to move forward with generating mice (Task 3) by the end of the year. Tasks 4 and 5 await the generation and characterization of the mice. Overall, the project is moving along as expected and there are no major problems to report. We eagerly await the knockout mouse, which will allow us to generate functional data.

## STATEMENT OF WORK

**Specific Aims 1 and 2: Role of the catenin p120 in Breast Cancer**

**Task 1:** Months 1 - 6. Generation of the knockin contruct designed to introduce Cre-lox sites into the introns flanking the sequence to be deleted.

A. Insert the 4.639 kb genomic p120 sequence containing exons 3 - 5 into pBS246 and sequence critical regions to verify absence of mutations.

B. Subclone the targeting arms (regions flanking exons 3 - 5) into sites flanking the Not I site in pBS to generate pBS-A/C.

C. Insert the pBS246 Not I cassette into pBS-A/C to generate the final targeting vector.

**Task 2:** Months 3 - 9. Generation of floxed p120 ES cell lines

A. Transfect ES cells with the Cre-loxP targeting construct, select transfectants in G418, and screen for homologous recombination by southern analysis.

B. Generate the appropriate recombination event by transient transfection of Cre recombinase, gancyclovir selection, and analysis by PCR.

C. Test the cell lines in vitro to verify the ability to induce p120 loss in the presence of Cre

**Task 3:** Months 9 - 15. Generate floxed p120 mice

A. Blastocyst injections by the Vanderbilt Transgenic Core and generation of several founder lines with germline transmission containing the heterozygous floxed p120 allele.

**Task 4:** Months 15 - 24. Generate homozygous $p120^{flox}$ / WAP-Cre mice
A. $p120^{flox}$ / + and $tg^{WAP\text{-}Cre}$ / + crosses to generate experimental and control mice.
B. Characterization of the resulting mice by PCR and southern blotting.

**Task 5:** Months 24 - 36. Determine the effects of p120 loss in normal and abberant breast function.
A. Determine effects of p120 loss on lobular-alveolar development and function.
B. Test the long term effects of p120 loss in mammary tumor progression and malignancy by crossing the $p120^{flox}$/WAP-Cre mice with TAg and/or MMTV-MT mouse models for mammary carcinogenesis.

**KEY RESEARCH ACCOMPLISHMENTS** (bullet format)

*Cloned and sequenced the relevant p120 genomic DNA between exons 1 and 8.
*Subcloned the sequenced DNA into a vector amenable to further manipulation.
*Inserted lox-P sites into introns flanking exons 1 – 7.
*Introduced frt sites on either side of the neo cassette.
*Ligated fragments of the above constructs to generate the targeting vector, containing floxed p120 sequences and a frt flanked neo cassette.

**REPORTABLE OUTCOMES**

This is a long term project requiring generation of a conditional knockout mouse. Although the project is proceeding on schedule, it is unlikely to result in reportable outcomes until the mouse is available. We anticipate completion of the mouse by next summer if the work goes as planned. I anticipate that at the end of this award, the availability of the p120 conditional knockout mouse, and preliminary data derived from this model, will be an excellent starting point for NIH RO1 funding to explore further the role of p120 and the E-cadherin complex in breast cancer and metastasis.

**CONCLUSIONS**

As described above, we have completed a significant body of work resulting in the floxed p120 targeting vector necessary to generate the conditional p120 knockout mouse. So far, we have made only minor modification to the original strategy and everything is proceeding as planned. By next year, we should have our mouse and begin functional experiments.

**REFERENCES:** none
**APPENDICES:** none